TREPONEMAL IMMOBILIZATION TEST IN EXPECTANT MOTHERS*

RESULTS IN 142 REACTORS TO STANDARD SEROLOGICAL TESTS

BY

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It is now established that the Treponemal Immobilization Test (TPI) can with great certainty distinguish between specific and non-specific positive reactions in the standard serological tests for syphilis (STS). The frequency of non-specific positive STS in different populations can thus be measured. Of considerable clinical importance is the proportion between specific and non-specific positive STS in a given population group; especially in presumably healthy people examined by routine STS, where positive serological tests may be the only means of establishing the diagnosis of latent syphilis.

The present communication deals with the results of STS and TPI tests in the routine antenatal examination of Norwegian women.

Material

The Sero-diagnostic Department, State Institute of Public Health, Oslo, examines annually about 40,000 antenatal blood samples. It covers the population of Southern and Eastern Norway, including Oslo. All samples are examined by the STS and Rhesus tests.

From April 1, 1957, to February 3, 1958, 31,562 antenatal sera from about 27,445 pregnant women were examined. (Those who are Rhesus-negative [about 15 per cent.] have two blood samples examined during each pregnancy.) 249 sera from 168 women (0.61 per cent. of all the women examined) gave positive reactions with one or more of the STS used. The TPI was carried out on 183 blood samples from 149 of these women. In the remaining nineteen STS-positive women, the TPI was not done because the amount of serum available was insufficient.

The extent to which the present series represents "all Norwegian pregnant women" may be partially illustrated by the fact that 64,171 living children were born in Norway in 1956, while in the same year routine antenatal testing was carried out on sera from about 49,800 pregnant women in the four laboratories which perform these examinations (personal communications). It is reasonable to assume that most of the women who were not examined were multiparae who had been found Rhesus-positive and STS-negative during previous pregnancies. The extensive use of routine antenatal testing makes our series fairly representative of at least the population of Southern and Eastern Norway. It should be noted that STS-testing and Rhesus-examinations of pregnant women in Norway are carried out on the same blood sample and in the same laboratory, so that no separate sample is necessary for the syphilis examinations. Furthermore, these examinations are carried out free of charge. These two features help to increase the effectiveness of routine STS-testing on pregnant women.

Methods

For syphilis examination the following three routine tests are used:

1. Meinicke Clarification Test II (MKR) (cardiolipin-lecithin-balsam of Tolu-antigen).
2. Bordet-Wassermann Complement-Fixation Test (WaR) (cardiolipin-lecithin-cholesterol-antigen).

Positive sera are titrated in MKR and WaR.

The technique for MKR titration is:

First tube, serum undiluted; second tube, dilution 1:2 (diluent; pooled MKR-negative serum); further doubling dilutions for each successive tube. Each tube is given a titration score according to the degree of clarification (4 = complete clarification, 0 = no clarification).

The technique for the WaR titration is:

First tube, dilution 1:5 (diluent; saline); then doubling dilutions as for MKR. Each tube is given a titration score according to the degree of haemolysis (4 = complete inhibition of haemolysis, 0 = complete haemolysis).

For both reactions the titre value is given as the sum of the titration scores; the titre values thus run roughly parallel to the logarithm of the reagin content in the serum.

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**TPI Technique.**—The test-tube contains the following:

STS-positive serum 0.05 ml.; undiluted pooled fresh guinea-pig serum 0.20 ml.; Treponema pallidum suspension 0.25 ml.

The reading is done after 18 hours. The test is counted as inconclusive if less than 70 per cent. of the treponemata in the control tube (containing inactivated guinea-pig serum) are alive. Each sample is tested at least twice, on different days. From August 22, 1957, a titration of "The WHO Reference Pool No. III for TPI Test" has been carried out on each TPI-testing day. Except in a few irregular cases the titres have been between 320 and 686 (average 516). The guinea-pig serum is filtered by suction through an asbestos filter*. This removes the numerous small particles normally seen in unfiltered serum, which otherwise often render the reading difficult. To the basic medium is added streptomycin (0.1 mg./ml.).

In most cases, serum for TPI-testing could not be taken from the specimen before the STS results were ready, i.e. 1 or 2 days after the specimen had arrived at the laboratory. During this time, the specimen tubes are left unstoppered, and the specimens are handled with non-sterile pipettes.

**Results**

(a) **STS Pattern†** compared with TPI Results

The TPI test was conclusive (positive or negative) in samples from 142 women. Of 102 women who gave positive reactions with the MKR or the WaR or both, 83 (81.37 per cent.) were TPI-positive and nineteen (18.63 per cent.) were TPI-negative. Of forty women who reacted to the hypersensitive W-Br. only, three (7.50 per cent.) were TPI-positive and 37 (92.50 per cent.) TPI-negative.

The TPI was inconclusive in twenty of the 183 sera examined (10.93 per cent.). From seven women who gave an inconclusive TPI reaction only a single blood sample could be procured; the remaining thirteen inconclusive sera were from women, who subsequently gave conclusive TPI results in additional blood samples.

A comparison of the WaR and MKR titres with the TPI result is given in Tables I and II (only the first sample from women who had several specimens examined, has been included in these Tables).

Two tendencies may be noted:

(1) A higher proportion of the presumed non-specific MKR reactions are of low titre; 35 (44 per cent.) of eighty MKR-positive sera which were positive with the TPI, had low titres of 1-5, whereas eleven (65 per cent.) of the seventeen MKR-positive sera which were negative with the TPI had equally low titres.

(2) All sera with high MKR titres also had positive TPI tests.

**Table I**

<table>
<thead>
<tr>
<th>No. of Cases</th>
<th>Titre Scores* in the Bordet-Wassermann Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>TPI-Positive</td>
<td>35</td>
</tr>
<tr>
<td>TPI-Negative</td>
<td>12</td>
</tr>
</tbody>
</table>

* See text under Methods

**Table II**

<table>
<thead>
<tr>
<th>No. of Cases</th>
<th>Titre Scores* in the Meinecke Clarification Test II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>TPI-Positive</td>
<td>3</td>
</tr>
<tr>
<td>TPI-Negative</td>
<td>2</td>
</tr>
</tbody>
</table>

* See text under Methods

These differences are, however, not statistically significant. Hence it may be stated that the distribution of WaR and MKR titres in the TPI-negative and the TPI-positive sera is similar. The most striking feature seen in the Tables is the pronounced difference between the WaR titres and the MKR titres within the group of TPI-positive sera. Of 83 STS-positive women, 35 were WaR-negative, but only three were MKR-negative. Furthermore, the number of women who gave positive MKR tests of low or moderate titres (titre-groups 1-5 and 6-10) greatly exceeds that of those who had positive WaR tests of the same titres. These figures clearly illustrate the well-known fact that, in cases of late syphilis, the WaR becomes negative before the MKR.

From April 1, 1957, onwards, the first sample from each of the 142 women constituted a group of sera in which the sensitivity and specificity of the three STS could be compared:

86 were TPI-positive, of which 80 reacted with the MKR, eighty with the W-Br., and 48 with the WaR.

Of the 142 sera, the WaR was positive in 55, of which seven (12.7 per cent.) were TPI-negative. The MKR was positive in 97, of which seventeen (17.5 per cent.) were TPI-negative. The W-Br. was positive in 131, of which 51 (38.9 per cent.) were TPI-negative.

† "STS Pattern" denotes the reactivity of the serum measured by the three tests (MKR, WaR, and W-Br.) simultaneously.
(b) Age compared with TPI Results

The women were divided into two major age groups: those born before, and those born after January 1, 1929 (Table III). The incidence of positive TPI tests was 67·35 per cent. in the older age group and only 45·45 per cent. in the younger age group. The difference is clearly significant (a 2×2 Table gives \( \chi^2 = 5·2, i.e. P = 0·02 \)). This difference may have arisen either through a higher incidence of STS-positive syphilis among the older pregnant women, or through a higher incidence of false STS-positives among the younger women.

<table>
<thead>
<tr>
<th>Year of Birth</th>
<th>TPI-Positive</th>
<th>TPI-Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1929–1941</td>
<td>20</td>
<td>24</td>
<td>44</td>
</tr>
<tr>
<td>1914–1928</td>
<td>66</td>
<td>32</td>
<td>98</td>
</tr>
<tr>
<td>Total</td>
<td>86</td>
<td>56</td>
<td>142</td>
</tr>
</tbody>
</table>

Table IV*, which shows the absolute incidence of STS-positive syphilis and presumed non-specific STS in the two age groups, suggests that the first explanation is probably correct. The incidence of STS-positive syphilis is 0·692 per cent. among the pregnant women born before January 1, 1929, but only 0·146 per cent. among those born after this date (a highly significant difference; \( P < 0·001 \)). The incidence of presumed non-specific reactions is also somewhat higher in the older group, but the difference is not significant (\( P = 0·19 \)).

<table>
<thead>
<tr>
<th>Year of Birth</th>
<th>Number of Women Tested</th>
<th>Positive STS and Positive TPI</th>
<th>Positive STS and Negative TPI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number</td>
<td>Per cent.</td>
</tr>
<tr>
<td>1929–1941</td>
<td>13,653</td>
<td>20</td>
<td>0·146</td>
</tr>
<tr>
<td>1907–1928</td>
<td>9,546</td>
<td>66</td>
<td>0·692</td>
</tr>
<tr>
<td>Total</td>
<td>23,199</td>
<td>86</td>
<td>0·371</td>
</tr>
</tbody>
</table>

Table V* shows the incidence of STS-positive syphilis in each of eight 5-year age groups.

* In Tables IV and V the total number of STS-tested women has been calculated as 27,445×\( \frac{142}{166} = 23,199 \). The age distribution was computed on the data from one-third of this number (i.e., 7,723). The seven women who gave inconclusive TPI results, as well as the nineteen who could not be tested, are presumed to constitute an "unselected" sample from the total of 168 STS-positive women.

(c) Duration of Positive STS compared with STS Pattern and TPI Results

In order to discover those cases in which a positive STS had already been recognized, and those in which positive reactions were detected for the first time during the experimental period, we consulted the "STS register", which comprises all individuals found STS-positive in this laboratory.

**TPI-Positive Women Reactive with the MKR or the W-Br. Only (Total 3).**—In one of these three women, one STS-positive blood sample was recorded before April 1, 1957, the earliest one in 1946. In four others confirmation of syphilitic infection was obtained from their physicians.

**TPI-Positive Women Reactive with the W-Br. Only (Total 3).**—In one of these three women, one STS-positive blood sample was recorded before April 1, 1957.

It appears that, of 86 syphilitic pregnant women, at least 61 (71 per cent.) were known cases; at most only 29 per cent. were diagnosed solely by routine antenatal testing during the experimental period.

**TPI-Negative Women Reactive with the MKR or the W-Br. Only (Total 19).**—In five women one or more STS-positive specimens were registered before April 1, 1957. The interval between the first and last STS-positive sample is about 1 year in two women, about 5 years in one, and about 7 years in the other two. These five women may thus be classified as "chronic biologic-false positive reactors".

**TPI-Negative Women Reactive with the W-Br. Only (Total 37).**—None of these were known to have positive STS reactions before the experimental
period. In no case did the positive W-Br. persist for more than 6 months. The positive non-specific W-Br. in these cases is obviously a transient reaction.

(d) Clinical Information

Inquiries about the 56 TPI-negative women were made by writing to their physicians, and replies were received from 32. All stated that the patient had neither history nor clinical evidence of syphilis and in eight cases the physician reported that the patient had been ill when the STS-positive blood sample was collected (four with “influenza”, two with anaemia, one with varicella, and one with hidrosadenitis).

Discussion

Of 102 pregnant women who gave positive reactions with the MKR or the WaR or both, one-fifth were false-positive reactors and four-fifths were syphilitics, judged by the TPI test. This frequency of non-specific reactors corresponds to that in the series of antenatal sera reported by Wilkinson and Sequeira (1955).

Our study shows:

1. That the W-Br. gives transient positive reactions in many non-syphilitic sera, confirming our previous impression of this test. This “hypersensitive” test should therefore not be used for routine screening.

2. That STS-positive syphilis is more frequent in older than in younger pregnant women.

3. That in most cases of STS-positive syphilis the presence of syphilis had been recognized before.

During and immediately after the Second World War the frequency of acquired syphilis rose steeply in Norway, predominantly in women, but this has since declined. The older pregnant women have therefore run more risk of exposure to syphilitic infection than the younger ones. The two age groups in Table IV represent approximately the group of women exposed to the “war syphilis”, and the younger group not so exposed. Analysis of 176,702 antenatal tests performed in this laboratory during the 6 years 1948 to 1953 (Vogt and Grelland, 1953) showed no certain variation of syphilis frequency with age, but at that time the “war syphilis” period was not far away.

An inconclusive TPI result was found relatively frequent in our material (in 11 per cent. of specimens), but our samples were not collected aseptically, and may accordingly have been more or less contaminated.

Most of the previously published TPI investigations of STS-positive sera are not comparable to our material, because they were selected, more or less, from “problem cases” (Bossak, Falcone, and Harris, 1957; MacPherson, Ledbetter, and Martens, 1955; Miller and Smith, 1953; Mohr, Moore, Nelson, and Hill, 1950; Thivolet and Rolland, 1956; Wheeler, Van Goor, and Curtis, 1954). The published reports of TPI examinations of STS-positive antenatal cases are few. Wilkinson and Sequeira (1955) found five TPI-negative among 29 STS-positive routine antenatal samples. They also examined 244 STS-positive antenatal “problem sera”, and found 27·5 per cent. TPI-negative. Further antenatal tests have been done by Wheeler, Van Goor, and Curtis (1954), who found 29 of 39 STS-positive reactors to be TPI-negative, and by Ranque, Tramier, Depieds, and Moignoux (1953), who found six TPI-negative cases in fifteen STS-positive reactors.

The results published by authors who have studied different groups of patients show considerable differences between the ratio of non-specific to specific positive STS reactions; but it is certain that non-specific positive reactions provide a considerable proportion of all positive reactions. This, with the fact that the strength or pattern of the positive STS reactions gives no clue to specificity in the individual case, leads to the conclusion that all STS-positive patients in whom syphilitic infection cannot be proved beyond doubt by clinical observation or by previous history should be examined by a TPI test. This is now done in our laboratory. Where lack of laboratory facilities prohibit full-scale TPI-testing, antenatal samples ought to be given priority.

Summary

In the Sero-diagnostic Department, State Institute of Public Health, Oslo, blood samples from about 27,445 unselected pregnant women were examined by the standard serological tests for syphilis (STS) during a period of 10 months (from April 1, 1957, to February 3, 1958). 168 women gave positive reactions with one or more of the STS used (Bordet-Wassermann (WaR) Meinicke II (MKR), and Wadsworth-Brown (W-Br.) tests). 149 of these were examined with the Treponema pallidum Immobilization Test (TPI).

102 women gave positive reactions with the MKR or the WaR or both. Of these, 81·37 per cent. were TPI-positive, and 18·63 per cent. were TPI-negative. Thus in the routine antenatal STS-testing in our laboratory non-specific positive reactions formed about one-fifth of the total positive reactions.

Nineteen TPI-negative women were positive with the WaR or the MKR or both; in five of these the positive reactions had persisted for 1 year or more.
The distribution of WaR and MKR titres in the group of TPI-negative sera was roughly equal to that in the TPI-positive group.

The "hypersensitive" W-Br test gave non-specific positive reactions in sera from 37 women, whereas only three of the syphilitic cases would have been detected solely by the W-Br. This test should accordingly be abandoned for routine purposes.

About 70 per cent. of the TPI-positive pregnant women were known to have syphilis; the diagnosis was established solely as a result of the routine antenatal testing during the experimental period in only about 30 per cent. of cases.

The ratio between non-specific and specific reactors is higher in the younger pregnant women than in the older ones, because of a higher frequency of STS-positive syphilis in the older age group. The total frequency of STS-positive syphilis in the cases tested was 0.371 per cent.

It is strongly recommended that the TPI test should be performed on all individuals who give positive STS without having definite proof of the disease especially pregnant women.

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REFERENCES